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Eurogin roadmap 2017: Triage strategies for the management of HPV-positive women in cervical screening programs

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Mini Review

Cervical cancer screening will rely, increasingly, on HPV testing as a primary screen. The requirement for triage tests which can delineate clinically significant infection is thus prescient. In this EUROGIN 2017 roadmap, justification behind the most evidenced triages is outlined, as are challenges for implementation. Cytology is the triage with the most follow-up data; the existence of an HR-HPV-positive, cytology-negative group presents a challenge and retesting intervals for this group (and choice of retest) require careful consideration. Furthermore, cytology relies on subjective skills and while adjunctive dual-staining with p16/Ki67 can mitigate inter-operator/-site disparities, clinician-taken samples are required. Comparatively, genotyping and methylation markers are objective and are applicable to self-taken samples, offering logistical advantages including in low and middle income settings. However, genotyping may have diminishing returns in immunised populations and type(s) included must balance absolute risk for disease to avoid low specificity. While viral and cellular methylation markers show promise, more prospective data are needed in addition to refinements in automation. Looking forward, systems that detect multiple targets concurrently such as next generation sequencing platforms will inform the development of triage tools. Additionally, multistep triage strategies may be beneficial provided they do not create complex, unmanageable pathways. Inevitably, the balance of risk to cost(s) will be key in decision making, although defining an acceptable risk will likely differ between settings. Finally, given the significant changes to cervical screening and the variety of triage strategies, appropriate education of both health care providers and the public is essential.

HPV testing for cervical cancer screening is now a reality with several countries, either adopting this modality at the program level or introducing it through the execution of regional pilot studies.[1] Support for this approach to screening is also endorsed by various professional societies and organizations which have global influence and reach.[2–5] While there is a wide and growing consensus that HPV molecular testing is the most accurate and cost-effective method of primary screening, there is a comparative lack of consensus regarding the optimal means of risk stratification or "triage" of primary HPV infections. This is evidenced by the heterogeneity of triage algorithms either proposed or applied across various settings[6] (Table 1). Given that most HPV infections follow a benign course, effective triage is crucial to ensure the screening participant is not subject to the burden of unnecessary follow-up and that resources are used efficiently. This point is further emphasized by the fact that compared to cytology based primary screening, HPV testing will generate significantly more screen "positives" in the initial round of screening.[7,8]

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Table 1. Active or proposed triage policies for HPV-based screening

Country	Screening policy[1]	First triage	Second triage	Reference
Australia/ New Zea-land	HPV alone	HPV16/18+: colposcopy hrHPV other: LBC: • if HSIL+: colposcopy • if ≤LSIL: second triage	At 12 M: hrHPV testing: • If +: colposcopy, • If −: routine screening	http://wiki.cancer.org.au/australiawiki/images/a/ad/National_Cervical_Screening_Program_guidelines_long-form_PDF.pdf
England (pilot study)[2]	HPV alone	Current **pilot** (6 sites) algorithm: cytology, if ASC-US+: colposcopy, if NILM: second triage. HPV16/18 typing also assessed in component of pilot sites where persistent 16/18 positivity at first and second triage = colposcopy	For pilot studies: At 12 M: hrHPV testing: • If +: colposcopy, • If −: routine screening TBD for roll out	https://www.gov.uk/government/publications/human-papillomavirus-hpv-primary-screening-protocol
Germany[3]	Co-testing	If ASC-H, AIS, HSIL: colposcopy, if NILM-LSIL: second triage.	At 6–12 M: co-testing • If + (any test): colposcopy • If −: routine screening	P. Hillemans, Department of Gynaecology and Obstetrics, Hannover Medical School; https://www.g-ba.de/institution/presse/pressemitteilungen/641/
Ireland[4]	HPV alone	Cytology, if ASC-US+: colposcopy, if NILM: second triage. Inclusion of limited typing likely but not yet defined.	To be determined.	Health Technology Assessment (HTA) of human papillomavirus testing as the primary screening method for prevention of cervical cancer. Health Information and Quality Authority. May 2017; https://www.hiqa.ie/sites/default/files/2017-05/HPV%20HTA%20technical%20report-%202605 2017_updated.pdf
Italy[5]	HPV alone	Reflex cytology, if ASC-US+: colposcopy, if NILM: second triage.	At 12 M: hrHPV testing: • If +: colposcopy, • If −: routine screening	http://www.gisci.it/documenti/convegni/firenze2014/workshop/carozzi_gisci_20140611.pdf
Netherlands	HPV alone	Cytology, if ASC-US+: colposcopy, if NILM: second triage.	At 6M: cytology • If ASC-US: Referral to colposcopy • If NILM: routine screening	http://www.britishcytology.org.uk/resources/Primary_HPV_screening_The_Dutch_experience.pdf
Norway[6]	HPV alone	Reflex cytology if ASC-US+: colposcopy. If reflex cytology = NILM second triage.	At 12 M: hrHPV testing • If +: colposcopy, • If −: routine screening	Ameli Trope & Mari Nygard, Norwegian Cancer Registry
Scotland	HPV alone	Cytology, if ASC-US+: colposcopy, if NILM: second triage.	At 12 M: hrHPV testing: • If +: colposcopy, • If −: routine screening	Tracey Curtis, National Services Division and Timothy Palmer, University of Edinburgh
Sweden[7]	HPV alone	Cytology, if ASC-US+: colposcopy	At 36M: cytology • If ASC-US: Referral to colposcopy If NILM: routine screening	http://www.socialstyrelsen.se/riktlinjer/nationellascreeningprogram/livmoderhalscancerscreeningme

As a growing number of countries prepare for HPV primary screening, the triage issue becomes particularly timely. This urgency was reflected at the EUROGIN 2016 meeting, where several of the scientific papers focused on the application of triage strategies. Consequently, a group of experts were tasked with using the vehicle of the now established "Eurogin roadmap" (the eleventh Roadmap release since 2007) to outline evidence, benefits and challenges

Table 1. Active or proposed triage policies for HPV-based screening (Continued)

Country	Screening policy[1]	First triage	Second triage	Reference
Turkey	HPV alone	HPV genotyping and evaluation of CP If cytology=ASC-US+: or if HPV16/18 regardless of cytology results: colposcopy. If HPV positive for other than HPV16/18 and smear is NILM: second triage.	At 3–6M: hrHPV testing. If hrHPV-: routine screening. If hrHPV+: genotyping and evaluation of CP with same algorithm as 1ry triage. If cyto=NILM and other hrHPV: third triage 3–6M later.	Turkey: M. Gultekin, Cancer Control Department, Public Health Institute, Ministry of Health, Ankara. ≤seurld≥http://kanser.gov.tr/Dosya/Kitaplar/turkce/Turkiye_Kanser_Kontrol_Program_ing.pdf≤/seurld≥
USA	Cotest	Immediate referral of HPV-positive ASC-US, any LSIL+HPV+/NILM second triage.	Repeat HPV/cytology. If either HPV+ or ASC-US+, referral to colposcopy If co-test negative, routine screening.	Saslow *et al.* (2012), Ref. 21
	HPV alone	HPV16/18+: colposcopy hrHPV other: reflex LBC, if >=ASC-US: colposcopy; if reflex LBC=NILM: second triage.	At 12M: repeat co-test. If either HPV+ or ASC-US+, referral to colposcopy If co-test negative, routine screening.	Huh *et al.* (2015), Ref. 2

Abbreviations: ASCUS, atypical squamous cells of undetermined significance; CP, conventional Pap smear; LSIL: low-grade squamous intraepithelial lesion; NILM: negative for intraepithelial lesion or malignancy.
[1]For a description of the details of the screening policies, see Wentzensen *et al.*[1]
[2]Final decision on first & second triage options for national roll out in England to be determined.
[3]Triage policy still needs national approval in Germany.
[4]Second triage test is not yet determined for Ireland.
[5]Cytology for women 25–33 years. HPV alone for women 34–64 years.
[6]Cytology for women 25–33 years. HPV alone for women 34–69 years.
[7]Cytology is offered to women 23–29 with HPV only interval = 3 y, age 30–49, with one co-test at age 41; HPV only, interval = 7 y, age 50–64. Non-participating continue to be invited yearly up to age of 70.

surrounding current triage strategies and to summarize some of the key technical developments in both sampling and diagnostics that may bear influence.

Cytology

Arguably, the strategy with the longest available follow up data is cytology. Cytological triage of HPV-positive women has been evaluated within three European randomized controlled trials, initially designed to compare HPV testing (alone or with cytology concurrently) to cytology screening over two screening rounds.[9–11]

Notwithstanding minor protocol differences across these trials, women with abnormal cytology were referred to colposcopy while the remainder were recalled to repeat an HPV test; alone or with cytology, after 6–18 months and referred to colposcopy in the case of persistent positivity (type specific in one study). All studies showed a significantly reduced occurrence of CIN3+ in the HPV arm at round 2. In addition, a pooled analysis of 4 studies with data on two screening rounds, including the three described, (and one without triage), found significantly less cancer in the HPV arm with no evidence of heterogeneity between studies. Conversely, the biopsy rate was similar between arms in the studies with cytological triage but higher in the trial without it.[12] Thus, cytological triage with HPV repeat-testing in cytology-negative women can be considered validated. In the aforementioned trials, cytology interpretation was blind to HPV result. In a Finnish trial (performed over one screening round), cytology was performed on HPV-positive women, with knowledge of HPV status. Interestingly, the detection of CIN was higher in the HPV arm even when considering only referrals due to reflex abnormal cytology.[13] This suggests that knowledge of HPV positivity may affect the performance of cytology. This observation is consistent with a substudy of the NTCC trial where HPV-positive Paps were dispatched for external cytology review to a laboratory blinded to information other than HPV status. The cross-sectional relative sensitivity for CIN2+ of HPV informed vs blind cytology was 1.58 (95% CI = 1.22–2.01).[14] A further important consideration is that as cytology relies on subjective morphological assessment, between-site variation in interpretation and performance can be expected.[14] In 2012, in ten Italian routine, local programs based on HPV testing, the proportion of HPV positive women judged to have ≥ASC-US varied from 20.0% to 56.9%.[15] This said, it should be noted that, as a consequence of the screening protocol applied (HPV+/cytologically normal women at baseline were referred to colposcopy if still HPV+ at 12 months), this variation had a

Table 2. Prevalence of carcinogenic HPV types as a proportion of HPV-positive samples in cervical cancers and CIN3 worldwide [genotype-specific positivity includes that contributed by multiple HPV infections [Adapted from Guan IJC 2012[18]]

HPV type	Invasive cancer ($n = 40,679$)	CIN3 ($n = 11,618$)
HPV16	64.7	54.5
HPV18	16.5	4.9
HPV58	5.5	10.8
HPV33	5.1	11.0
HPV45	4.3	1.7
HPV52	3.7	10.9
HPV31	3.5	10.7
HPV39	1.5	1.5
HPV59	1.3	0.8
HPV35	1.2	3.1
HPV56	0.9	1.5
HPV51	0.8	3.5
HPV68	0.6	1.1
Multiple	11.9	15.8

relatively small impact on the colposcopy referral rate, at baseline or at 12 months (only a 4.2% increase for a 10% increase in immediate referral).[16] However, the total number of colposcopies is also dependent on the follow-up guidelines of women with a negative colposcopy.

More generally, the above data show that when judging the performance of any triage approach, the entire process should be taken into account, rather than just the immediate triage test. Improvements to immediate triage tests can be fully exploited only if the interval to retesting is considered carefully. Finally, a practical consideration of cytology-triage is that it requires a highly skilled workforce and significant investment in ongoing quality assurance to perform optimally. This is possible and has precedent in high-income countries with the relevant infrastructure, yet even in this context, the increasing move to HPV primary screening will bring about sizeable reductions in the overall cytology workload. Adequate recruitment, training and retention of cytology staff in an era of HPV primary screening may prove challenging and efforts to address this are required. A novel approach to automated cytology that achieves performance similar to expert manual evaluation may serve as mitigation to these challenges.[17]

HPV Genotyping

Current HPV tests include 13–14 HPV genotypes that vary substantially in their association with cervical cancer and precancer (Table 2). The variation in risk has prompted development of genotyping strategies for triage purposes. In cervical cancer, by far, the most important type is HPV16, followed by HPV18. However, there is some regional variation of type prevalence that could affect which types are included in screening and triage assays.[18] While HPV16 is also dominant in CIN3, HPV31, 33, 52, and 58 are more common than HPV18 (Table 2). There are several important considerations that affect HPV genotyping-based triage: (*i*) Detection of a specific HPV genotype cannot differentiate between a transient infection and a prevalent precancer. (*ii*) HPV genotype detection predicts risk of precancer over many years. Thus, women may be at increased risk of future precancer, but they may not have any detectable lesions. (*iii*) Deciding which types to include in a triage genotyping assay must balance the prevalence of the type in disease and in the healthy population, measured by the absolute risk of disease related to genotype. These points demonstrate that unavoidably, there is some unnecessary colposcopy referral with genotyping. Table 3 shows the absolute risk of CIN3+ and CIN2+ for genotypes in a large population of HPV-positive women. Importantly, the ranking of types may differ depending on chosen endpoint; CIN3+ is generally preferable as many CIN2s regress spontaneously.[19] Several commercial HPV assays, some of which have been FDA-approved and or clinically validated via other means offer partial genotyping, typically measuring at least HPV16 and HPV18.[20] Current US guidelines recommend immediate referral of HPV16/18-positive women with normal cytology to colposcopy in a HPV-cytology co-testing strategy.[21] A recently FDA-approved strategy, endorsed by interim expert consensus, recommends immediate referral of HPV16/18-positive women, with cytology triage of women positive for other HR types.[2] Furthermore, recent data from the US show that extended genotyping (up to nine types or combinations) combined with cytology can provide refined risk-stratification through the identification of type-specific persistence.[22]

Table 3. Absolute risk of CIN3+ and CIN2+ for genotypes and genotype combinations in HPV-positive women from Kaiser Permanente Northern California

Type or type combination	CIN3+ absolute risk	CIN2+ absolute risk
16	22.5	35.6
18	11.7	20.7
31	8.5	20.7
33/58	8.5	18.8
45	6.1	11.1
52	5.8	16.2
51	2.7	8.9
39/68/35	2.1	8.1
59/56/66	1.5	5.6

±p16 ± Ki67

p16INK4a (or p16) is a cellular protein which highlights disruption of the retinoblastoma (RB)/E2F pathway related to activity of the HPV oncogene E7. The diagnostic application

is through inmmunocytochemistry (or histochemistry), initially as a single marker and now as a dual stain with Ki67 (a proliferation marker which confers additional specificity).[23–26] While p16 staining still requires a level of morphological interpretation, it can reduce pattern-complexity by allowing focus on a small subset of p16-stained cells. In contrast, for the dual stain, the criterion for positivity is a single cell with a simultaneous brown cytoplasm (p16) and a red nucleus (Ki67). The longitudinal accuracy of p16 immunostaining (without Ki67) as a triage of HPV-positive women was studied within the NTCC trial.[27] In women 35–60 years, the risk of CIN3+ at 3 years was 4.7% among HPV+/p16+ women compared to 0.8% in HPV+/p16− women. Furthermore, 83.7% of women who had a CIN3+ at follow-up were p16+ at baseline. The authors concluded that HPV+/p16+ women warrant immediate colposcopy, whereas HPV+/p16− women could defer follow-up for at least 2 years. More recently, three studies have reported on dual-stained cytology as a triage of HPV-positive women, within primary screening cohorts,[28–30] all of which have indicated that dual-staining may enhance the sensitivity of cytology. The largest ($n = 7727$) was nested into the Athena trial[29] and showed that for CIN3+ detection, sensitivity of dual-stained cytology versus Pap cytology was significant higher (74.9% vs. 51.9%), as was NPV and PPV; whereas specificity was equivalent. Immediate colposcopy referral of all HPV16/18+ women combined with dual-stained cytology of women positive for non-16/18 genotypes provided the highest sensitivity for CIN3+ (86.8%). Additionally, a recent Scottish study showed higher sensitivity but lower specificity of dual stained, compared to conventionally stained, cytology.[31] It has been argued that the dual stain can reduce the requirement for "expert" cytology through simplifying interpretation and may improve interoperator variability compared to traditional cytology.[32] However, a recent study showed important differences in interobserver reproducibility according to a laboratory's experience highlighting the need for robust training and quality assurance.[33] In summary p16/ki67 dual stain is a credible tool for risk stratification of HPV-positive women and compares favorably to cytology.[34] Head-to-head comparisons now need to consider the cost effectiveness of this strategy compared with other stand-alone and combination options.

Cellular and viral methylation assays for triage of HPV infection

Methylation has a fundamental role in the development and outcome of malignancies and can be measured accurately and easily by automated methods. Approximately 10 human genes have consistently elevated methylation in cervical precancers and hypermethylation in most cancers, hence the appetite to focus on methylation targets for triage purposes.[35,36] Of prominence are *CADM1, EPB41L3, FAM19A4, MAL, miR-124, PAX1, and SOX1*. Methylation of certain HPV genes including L1 are also associated with precancer and invasive disease, especially for types HPV16, HPV18, HPV31, HPV33, and HPV45.[37,38] Table 4 shows the performance of human and HPV gene classifiers as triage tests for cervical precancers.[39] *MAL and CADM1* have been investigated extensively in hrHPV+ women; in one study of a screening population with a precancer and cancer endpoint

Table 4. Performance characteristics of selected DNA methylation studies in exfoliated cervical cells for CIN2+. The combination tests typically used any gene marker as positive, but see ref. 37 for specific details

Genes	Sensitivity %	Specificity %	PPV %
C13ORF18JAM3	74	76	NA
ANKRD18CP			
FAM19A4	70	66	55
EPB41L3	74	65	28
HPV16/18/31/33			
EPB41L3	90	49	51
HPV16/18/31/33			
CADM1	89	50	52
MAL			
miR-124			
PAX1	44	95	83
PCDHA4	75	80	73
PCDHA13			
DLX1	77	86	NA
ITGA4			
RXFP3			
SOX17			
ZNF671			
CADM1	62	78	49
MAL			
JAM3	55	90	NA
EPB41L3	60	57	NA
TERT	62	62	NA
FAM19A4	69	70	42
MAL	72	49	29
Mir-124			
CADM1	48	81	NA
MAL			
miR-124			
JAM3	65	77	NA
TERT			
EPB41L3			
C13ORF18			
CADM1	84	52	25
MAL			

(collectively CIN2+), these genes gave a sensitivity of 84% (95% CI 72–93), specificity of 52% (95% CI 48–57), and AUC of 0.72.[40] In the same set of women, the sensitivity and specificity of cytology were 66% (50–79%) and 79% (74–83%), respectively.[41] More recent studies on screening and colposcopy populations using a variety of human gene targets showed sensitivity ranging from 69% to 74% with specificity (for CIN2+) ranging from 66% to 76%.[39] A more comprehensive approach is to test for methylation of HPV *and* human genes. For example, a combination of *DAPK1 and* HPV on samples from a US colposcopy population gave a sensitivity of 80% and a specificity of 89% for CIN2+. Another study on a combination of *EPB41L3* and HPV in a UK colposcopy population gave a sensitivity of 90%, specificity of 49%, AUC = 0.82.[39] Given that performance in a colposcopy population does not necessarily translate to performance in a screening population the *EPB41L3*-HPV gene combination was validated in a separate study of hrHPV-positive women from a screening population in the UK and gave a sensitivity of 74% (59–85%), specificity of 65% (60–70%), and AUC = 0.78 (Table 4).[42] In comparison, HPV16 and HPV18 genotyping on the same samples had a significantly poorer performance as a triage ($p < 0.0001$). Methylation testing is still in the early stages but is showing good promise as an accurate molecular classifier. Technical improvements will likely improve clinical performance and can be expected in the next 5 years. Furthermore, even if methylation testing can deliver equivalent (rather than improved) performance compared to robust cytology, there are still positive aspects to this method including objectivity, consistency and applicability to automation and self-taken samples.

Special considerations: Triage of self-collected specimens

Offering self-sampling of cervico-vaginal material for HPV testing (HPV self-sampling) is an effective tool to increase screening coverage.[43] Moreover, HPV testing on self-samples is as accurate as on clinician-taken samples if target-amplification assays are used.[44] However, as cytology is not reliably applicable to self-taken samples,[44,45] offering cytology as a triage of women positive on their self-sample would necessitate a clinic visit. This confers a significant risk of loss to follow-up yet the issue would be circumvented if the triage test could be applied directly to the residual self-sample. In contrast to microscopy-based assays, molecular tests do not require the preservation of intact cells and may be used directly. The detection of (hyper) methylation of host cell genes by quantitative methylation-specific PCR (qMSP) on both self-collected vaginal lavages and brushes is feasible and shows promise as a triage.[46–50]

A recent RCT where qMSP for host genes *MAL* and *miR124-2* was compared to cytology (on an additional smear) as a triage of HPV positive self-samples, demonstrated that the qMSP was noninferior to cytology for the detection of CIN2+.[51] While the clinical performance of the qMSP did not exceed that of cytology, the logistic advantages/efficiencies of being able to apply the screening and triage assay to the same sample are clear. There is also evidence to suggest that certain methylation markers have the advantage of giving a very high reassurance for absence of cervical cancer or advanced CIN2/3 lesions with a high short-term progression risk in test negative women.[51,52] Finally, as with the other markers considered in this roadmap, it is perhaps prudent not to consider methylation biomarkers as only applicable in isolation. A study in which the performance of the *FAM19A4* and *miR124-2* methylation biomarker was assessed[52] showed that the addition of HPV16/18 genotyping results increased the sensitivity for CIN2+ significantly. The combination approach of 16/18 genotyping with methylation has parallels with combining cytology with HPV16/18 genotyping, with the former offering a pathway to full molecular screening and triage.

The role of next generation sequencing and viral genomics

Advances in next-generation sequencing (NGS) technologies have enabled the sequencing of HPV whole-genomes in a high-throughput, cost-efficient manner. The large-scale study of HPV genome variability will advance a deeper understanding of HPV biology and mechanisms of HPV carcinogenicity, which may help to improve the design of triage tools in the future. NGS has already generated important information about carcinogenesis and natural history of HPV.[53–55] For example, it has demonstrated that HPV 16 sublineages confer differential risks for disease as well as different tropisms for morphological lesion-type.[54,56–71] Among 3,215 HPV16-positive women in the US, the HPV16 A4, D2, and D3 sublineages conferred significantly increased risks for glandular lesions compared to the more common A1/A2 sublineages.[54] An international study of invasive cancers also found an enrichment of specific lineages in adenocarcinomas.[68] A triage test incorporating detection of a variants specific for adenocarcinoma could be used to enhance the detection of glandular lesions. At the level below a sublineage, there is an HPV isolate which is a genome differing by $\geq$2 nucleotides from all others. NGS has shown that thousands of unique HPV16 isolates exist.[55]

Furthermore, transient HPV16 infections have been shown to have a higher number of single nucleotide polymorphisms compared to transforming infections.[55] Notably, the strict genetic conservation of E7 was associated with a greater risk of HPV16-driven carcinogenesis.[55] The level of genetic variation in specific regions of the viral genome is important and could inform the design of future triage tools. Finally, given that NGS is capable of rapid sequencing of both host and viral genes, the technology lends itself to identifying several potential biomarkers of significance to infection and precancer concurrently such as HPV genotyping detection, variant classification and detection of somatic mutations.

Text Box 1
Elements to consider in the development of new generation of HPV tests appropriate for use in LMIC

- Small collection volumes are cheaper and make storage, transport and disposal of plastics and fluids easier; systems designed a priori for cytology are wasteful.
- Readily available simple blood collection tubes could be used for sample collection.
- Self-collected vaginal specimens are more acceptable than clinician-collected specimens to women and especially in cultures where gynecological examination requires a husband's permission.
- Tampon-like collection devices are easy to use, favored by women in certain settings and may be able to be transported without fluid.
- HPV tests should be cheap, simple to do and reproducible by all levels of healthcare providers with short turnaround time (<2 hr).
- Point of care testing by clinic staff would enable "screen and treat" programs where women could complete the screening process in a single visit.

Low and middle income countries (LMIC)

Cervical screening in LMIC is clearly associated with very different challenges from screening in high-income countries (HIC). WHO Guidelines recommend visual inspection with acetic acid (VIA) for population-based screening or HPV testing if it can be afforded.[3] However, most current HPV tests are designed for HIC and a new generation of tests which address the challenges in LMIC are needed (Text Box 1). Unless these issues are addressed, both primary HPV testing and associated triage could remain largely HIC issues. Options for triage in LMIC are associated with particular challenges. The lack of cytologists, pathologists and associated quality assurance usually means cytology-based protocols cannot be contemplated. Effective triage in a single "screen and treat" visit would overcome the challenges of follow-up and restricted opportunities for intervention in LMIC, but requires a simple, quick, affordable and objective biomarker test performed on the same sample as the primary screen.

Neither limited genotyping nor current biomarkers would satisfy these conditions. Work in rural Malawi has shown that trained staff who maintain a regular workload and adequate continued professional development can use VIA directly to differentiate treatable lesions and suspicious/advanced cancers[72,73] but that prior knowledge of HPV results can aid judgment and inform clinical management: Beatrice Kabota (personal communication). Sending all HPV-positive women for immediate treatment will result in over treatment, whereas restricting VIA to HPV positives may well entail a significant loss in sensitivity yet could address problems of capacity and retention of competent VIA providers. Inevitably, the tradeoff between resources available and program outcomes needs to be assessed country by country and this is an area of great interest as outlined in recent reviews.[74,75] Many LMIC have not implemented HPV primary screening due to the cost and challenges identified, whereas roll-out of a VIA service, while potentially more practicable is challenged by the need for ongoing quality assurance and monitoring. Use of <3 doses of HPV vaccine together with GAVI pricing have greatly advanced the potential for LMIC to consider national programs.[76] A combination of vaccination of adolescent girls and VIA for adult women may provide a better approach than HPV primary screening and an appropriate, as yet unidentified triage test although more evidence is needed to support this. To this end, projects built around the HPV FASTER recommendation [(of extending routine vaccination programs to women of up to 30 years of age (and to the 45–50-year age groups in certain settings)], paired with at least one HPV screening test at age 30 years will provide important data.[77]

Triage for immunized women

The impact that HPV immunization programs have conferred on both infection and disease is now clear at the population level in several countries.[78] The evidence of herd immunity and the potential utility of even one dose of vaccine adds to these encouraging observations, for the countries that can afford immunization programs.[79,80] However, immunization does present certain challenges for screening. The predictive value of any screening test will be influenced by the level of disease in the population.[81] Furthermore, the fraction of HR-HPV infections in vaccinated populations attributable to 16/18 will be significantly lower; in a recent study of females immunized aged 12–13, over 90% of residual HR-HPV infections at age of first screen were not HPV 16 or 18.[82] As a wealth of evidence demonstrates that non-16/18 HR-HPV types confer a lower risk for CIN2+, the PPV of HR-HPV screening may reduce so appropriate triages are even more relevant in vaccinated populations.[1] Limited genotyping in this population is likely to have diminishing returns given the scarcity of 16/18-positive infections and it is arguable that nonviral targets may be more appropriate or assays that offer genotyping beyond HPV 16/18. There have been few assessments of cytology triage specifically in immunized women (with or without adjunctive staining). Evidence

of deterioration in the predictive value of cytology as a primary screen in immunized women has been documented, although this does not translate into poor performance as a triage of HR-HPV-positive samples.[83] It is also worth noting that the extent of triage in immunized populations will reduce given evidence from modeling studies that indicate 10-year screening starting aged 30 is optimal.[84,85] Conclusions from modeling endeavors, while extremely helpful, clearly incorporate various assumptions and differing levels of vaccine-uptake, type of vaccine and dimensions of program (including the detail of catch-up immunization) will exert influence. This makes a comprehensive, "one size fits all" solution difficult.[1] In line with this, one of the recommendations from a recent Italian conference on screening for vaccinated women was that modifications to the status quo should only be imposed on women vaccinated routinely rather than as part of a catch-up program.[86] Furthermore, offering optimal screening and triage to increasingly mixed populations of immunized and unimmunized women, without creating impractical algorithms, and conflicting public-facing message(s) is a key challenge facing the community.

Appropriate educational initiatives

The transition from cytology to HPV-based screening presents unique challenges for patients and providers. With HPV screening, receipt of positive findings may result in a shift in the clinical discussion from an oncologic to a communicable disease approach. Despite the fact that HPV is the most prevalent STI worldwide,[87] there is a lack of awareness about HPV among the general population.[88,89] Women may feel anxiety, or shame when informed they are HPV positive.[90] Clinicians often feel ill-equipped to manage the questions of HPV-positive women.[91] These concerns could ultimately affect acceptance of a superior screening technology if implementation is not carefully planned, irrespective of what the subsequent triage may be. Key messaging should be developed to address the concerns around an HPV-positive result: destigmatize HPV by emphasizing the high prevalence; highlight HPV positivity does not indicate a woman has or will develop cervical cancer; ensure women are aware HPV may have been present for years, and does not reflect partner infidelity or promiscuity; move the focus of HPV testing from "STI identification" to an enhanced test for cervical cancer prevention. A key indicator of a woman's intention to receive HPV testing is endorsement by her care provider[92]; therefore, clinicians play a significant role alleviating distress by being prepared for the concerns and questions women will have. Cytology has been the primary cervical screening tool for decades. As a result, programs must invest appropriate time and resources to plan for HPV-based testing irrespective of the triage used. Without such investment, lack of engagement from both patients and providers could jeopardize successful acceptance, resulting in decreased attendance for screening or increased health system costs due to non-compliance with extended screening interval guidelines.

Conclusions

The choice of appropriate triage strategy for HPV-positive women is one of the key issues facing the cervical-screening community at present. As outlined above, there are various options associated with varying levels of evidence that either exploit considerable, existing expertise in morphological assessment or take advantage of recent developments in molecular technologies. Currently, there is no single approach which offers a binary solution of referral to colposcopy for the positives and routine recall for the negatives, with the HPV-positive-triage-negative group (whether by cytology, typing or methylation markers) representing a challenge. Technical refinement of molecular approaches and/or the appropriate combination of more than one option, either concurrently or in a stepwise fashion may deliver benefits provided the complexity and cost are not prohibitive. Defining an acceptable level of risk is also important to help calibrate triage tools to appropriate performance standards at the population level. For European settings, it has been proposed that triage-positive women should have (minimum) 20% risk of CIN2+ to indicate colposcopy, whereas triage-negative women should have a risk of <2% of CIN2+ to indicate routine recall.[93] These thresholds are lower in the US.[94] Longitudinal data from national HPV primary screening programs, where alternative triage options have been used will continue to be important and should ideally be stratified by vaccination status. Finally, should there be a move to an entirely molecular option for cervical screening, possibly in combination with self-sampling, communications around this paradigm shift must be managed carefully to ensure informed engagement and sustainable uptake.

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